BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problems Mailbox.

Access DB# 94050

SEARCH REQUEST FORM

Scientific and Technical Information Center

Requester's Full Name: Misook Yu Examiner #: 79031 Date: 5/13
Art Unit: 1642 Phone Number 30 8-2457 Serial Number: _____
Mail Box and Bldg/Room Location: _____ Results Format Preferred (circle): PAPER DISK E-MAIL

If more than one search is submitted, please prioritize searches in order of need.

****

Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: _____

Inventors (please provide full names): _____

_____

Earliest Priority Filing Date: _____

***For Sequence Searches Only* Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.**

Search sale of Caspase Assay kits

****

STAFF USE ONLY	**Type of Search**	**Vendors and cost where applicable**
Searcher: P. Schwebel	NA Sequence (#)_____	STN _____
Searcher Phone #: 308-4292	AA Sequence (#)_____	Dialog _____
Searcher Location: CM1 6A03	Structure (#) _____	Questel/Orbit _____
Date Searcher Picked Up: _____	Bibliographic ✓	Dr.Link _____
Date Completed: 5/13	Litigation _____	Lexis/Nexis _____
Searcher Prep & Review Time: 11	Fulltext _____	Sequence Systems _____
Clerical Prep Time: _____	Patent Family _____	WWW/Internet ✓
Online Time: 41	Other _____	Other (specify)_____

PTO-1590 (8-01)

ApoAlert™ Antibodies & Inhibitors

High-quality reagents for apoptosis research

- Study roles of proteases in apoptosis
- Induce apoptosis in human and mouse cells
- Detect apoptosis by cleavage of PARP

CLONTECH offers a wide variety of ApoAlert reagents that allow you to detect apoptosis at various stages using cell surface, nuclear, and cytoplasmic markers. In addition, antibodies available for both induction of apoptosis and Western blot analysis. We also offer reversible irreversible protease inhibitors.

PARP Monoclonal Antibody

Poly (ADP-ribose) polymerase (PARP) is a substrate for CPP32, a member of the interleuk converting enzyme (ICE) family of proteases, also known as caspases (1-5). The PARP Monoclonal Antibody can be used to detect PARP cleavage, and thus provides a useful ma for early apoptosis. Figure 1 shows a Western blot probed with the PARP Monoclonal Antib In cells treated with etoposide (Lane 2), the antibody detects only the 85-kDa cleavage fragment, indicating that the majority of cells are apoptotic. In contrast, in uninduced cells (L 1), the 116-kDa native PARP is the major form; here, the 85-kDa fragment is due to the bas of apoptotic cells normally present in cell cultures.

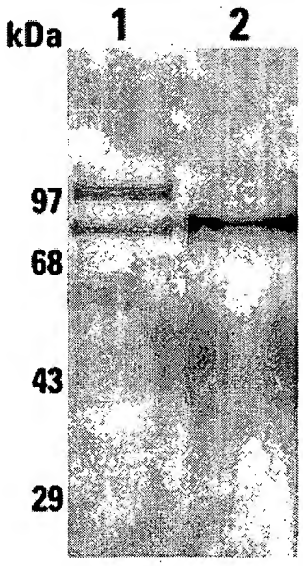

Figure 1. Western blot analysis with PARP Monoclonal Antibody reveals apoptosis. HL-60 cells were trea with etoposide to induce apoptosis. Cell lysate samples were electrophoresed on a 12% SDS/polyacrylamide ge electroblotted onto a PVDF membrane. The blot was incubated with a 1:1,000 dilution of PARP Monoclonal Anti followed by a secondary antibody conjugated to alkaline phosphatase. Lane 1: uninduced cells. Lane 2: induced cells.

Anti-Fas mAbs induce apoptosis

Fas, also known as CD95 or Apo-1, is a member of the tumor necrosis factor/nerve growth factor receptor family of cell-surface molecules. Stimulation of Fas by treatment with either

specific ligand (FasL) or anti-Fas antibodies induces apoptosis in lymphocytes and other ce types (6-9). Human Fas Monoclonal Antibody (IgG, Dx2) and Mouse Fas Monoclonal Antib (RMF2) can be used to study Fas-induced apoptotic pathways.

ICE-family protease inhibitors

For studying the effects of ICE-family proteases, you can choose from four ApoAlert Inhibito These synthetic, cell-permeable peptides are noncleavable analogs of ICE-family protease substrates. YVAD-CMK inhibits the activity of ICE protease. DEVD-CHO, DEVD-FMK, and VAD-FMK inhibit the activity of CPP32 protease; VAD-FMK may also inhibit the activity of o ICE-family proteases. Inhibition by DEVD-CHO is reversible, whereas YVAD-CMK, DEVD- and VAD-FMK are irreversible, noncompetitive inhibitors. Figure 2 shows the effects of DEVD-CHO, DEVD-FMK, and VAD-FMK on CPP32 protease activity.

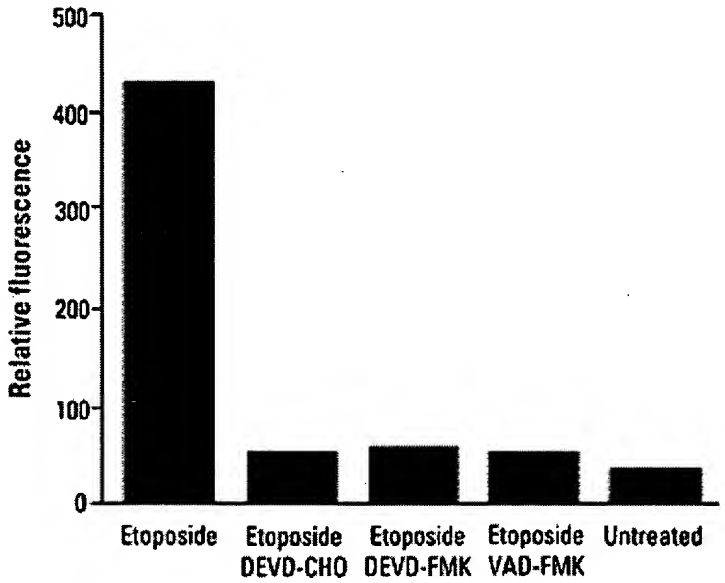

Figure 2. ICE-family protease inhibitors greatly reduce CPP32 activity. 32D cells were treated with 100 µM etoposide to induce apoptosis. 1 x 10^6 cells were preincubated with or without the indicated inhibitor at 37°C for CPP32 protease activity was measured using the ApoAlert CPP32 Fluorescent Assay Kit (#K2026-1, -2) accordi the User Manual. Results were analyzed using a fluorescence multiwell plate reader with a 360-nm excitation filt and a 508-nm emission filter.

Product	Size	Cat
ApoAlert CPP32 Inhibitor, DEVD-CHO	100 µl	8170
ApoAlert CPP32 Inhibitor, DEVD-FMK	100 µl	8172
ApoAlert ICE Inhibitor, YVAD-CMK	100 µl	8171
ApoAlert ICE-Family Protease Inhibitor, VAD-FMK	100 µl	8173
Human Fas Monoclonal Antibody (IgG, Dx2)	100 µg	8150
Mouse Fas Monoclonal Antibody (RMF2)	100 µg	8148
PARP Monoclonal Antibody (IgG1, C-2-10)	50 µl	8192

References

1. Yuan, J., *et al.* (1993) *Cell* **75**:641-652.
2. Fernandes-Alnemri, T., *et al.* (1989) *J. Biol. Chem.* **269**:30761-30764.
3. Tewari, M., *et al.* (1995) *Cell* **81**:801-809.
4. Kaufmann, S. H., *et al.* (1993) *Cancer Res.* **53**: 3976-3986.
5. Lazebnik, Y. A., *et al.* (1994) *Nature* **371**:346-347.
6. Yonehara, S., *et al.* (1989) *J. Exp. Med.* **169**: 1747-1756.
7. Suda, T. & Nagata, S. (1994) *J. Exp. Med.* **179**: 873-879.
8. Ogasawra, J. R., *et al.* (1993) *Nature* **364**:806-809.
9. Rouvier, E., *et al.* (1993) *J. Exp. Med.* **177**:195-200.

An Acrobat version of these *CLONTECHniques* pages is also available.

Please send us your comments or suggestions.

BD
1 Becton Drive
Franklin Lakes, NJ USA 07417

BD Biosciences Clontech
1020 East Meadow Circle
Palo Alto, CA 94303
877.232.8995, orders@clontech.com

The information included in online CLONTECHniques articles is the same as the printed version. These articles contain dated information and may not reflect CLONTECH's current product line. For the most current product information, please visit our Online Catalog or contact our Customer Service Department.

CLONTECHniques Oct 1999

Volume XIV, No. 4

CLONTECHniques is published quarterly in January, April, July, and October, by CLONTECH Laboratories, Inc., 1020 East Meadow Circle, Palo Alto, CA 94303-4230, USA

TOP

Trademarks

Trademarks The following are registered trademarks of CLONTECH Laboratories, Inc: Advantage®, ApoAlert™, Delta™, Living Colors™, MTN™ & TALON™.

The following are trademarks of CLONTECH Laboratories, Inc: AdvanTaq™, AdvanTaq Plus™, Atlas™, AtlasImage™, Atlas *Select*™, AtlasInfo™, CalPhos™, CHROMA SPIN™, ClonCapture™, CLONfectin™, CLONTECH™, CLONTECH PCR-Select™, Diversify™, ExpressHyb™, GC-Melt™, Great EscAPe™, λTriplEx2™, Mercury™, MTC™, MTE™, Premium RNA™, PRO™, PROTet™, pTet-Off™, pTriplEx2™, RevTet-Off™, RevTet-On™, TALONspin™, Tet-Off™, Tet-On™, Transformer™ & YEASTMAKER™.

Axioskop® is a registered trademark of Carl Zeiss, Inc. FPLC® & Superose® are registered trademarks of Amersham Pharmacia Biotech AB. GenBank™ is a trademark of International Biotechnologies, Inc. MetaMorph® is a registered trademark of Universal Imaging Corp. NucleoBond®, NucleoSpin® & NucleoTrap® are registered trademarks are trademarks of Macherey-Nagel GmbH & Co. Omega® is a registered trademark of Omega Optical, Inc. Superflow™ is a trademark of Sterogene Bioseparations.

TOP

CLONTECHniques

EDITOR
Stephanie Trelogan

CONTRIBUTING EDITORS
Amy Adams
Nancy Correll
Halina Duraj
Nancianne Knipfer, Ph.D.

GRAPHIC PRODUCTION
Jeff Baughn

PRODUCTION COORDINATOR
Marion Kerr

WEB PRODUCTION
Jeff Schwartz
Maureen Cyrot

BD
1 Becton Drive
Franklin Lakes, NJ USA 07417

BD Biosciences Clontech
1020 East Meadow Circle
Palo Alto, CA 94303
877.232.8995, orders@clontech.com

CLONTECH HOME | NEWS | PRODUCTS | SUPPORT | LITERATURE | ABOUT
international | site map | ordering | contact us | vector | user manuals | careers

PRODUCTS
- eCatalog
- Product Families
- Technologies
- New Products
- Ordering
- Sales

NEWS
- Special Offers
- Clontechniques
- Highlights
- Coming Soon
- Conferences

SUPPORT
- User Manuals
- Vector Information
- Bioinformatics
- Citations
- Application Notes
- FAQs
- Email Tech Support

LITERATURE
- Printed Literature
- Brochures
- Clontechniques
- Order Forms
- Product Lists

ABOUT
- Bd Biosciences
- Contact Us
- Employment

The information included in online CLONTECHniques articles is the same as the printed version. These articles contain dated information and may not reflect CLONTECH's current product line. For the most current product information, please visit our Online Catalog or contact our Customer Service Department.

CLONTECHniques April 1999

CLONTECHniques is published quarterly in January, April, July, and October, by CLONTECH Laboratories, Inc., 1020 East Meadow Circle, Palo Alto, CA 94303-4230, USA

CLONTECHniques

Kimberly Gasuad
Leila Hebshi
Nancianne Knipfer,Ph.D.
Eric Machleder
Valeria Natalie, Ph.D.
Nikki Zahl

Stephanie Trelogan

Jeff Baughn

Marion Kerr

Jeff Schwartz

Trademarks

The following are registered trademarks of CLONTECH Laboratories, Inc: Advantage™, ApoAlert™, Living Colors™, MTN™ & TALON™.

The following are trademarks of CLONTECH Laboratories, Inc: AdvanTaq™, AdvanTaq Plus™, Atlas™, AtlasImage™, AtlasInfo™, CalPhos™, ClonCapture™, ClonCapture-Ready™, CLONfectin™, CLONTECH™, CLONTECH PCR-Select™, Delta™, EcoPack™, ExpressHyb™, Great EscAPe™, Label-ON™, Master Blot™, Mercury™, MTC™, MTE™, Premium RNA™, pRevTet-Off™, pRevTet-On™, pTet-Off™, pTet-On™, QUICK-Clone™, RetroPack™, Retro-X™, RevTet-Off™, RevTet-On™, SMART™, TALONspin™, Tet-Off™ & Tet-On™.

Axioskop® is a registered trademark of Carl Zeiss, Inc. MetaMorph® is a registered trademark of Universal Imaging Corp. MicroMAX is a product of Roper Scientific, formerly Princeton Instruments. NucleoBond®, NucleoSpin® & NucleoTrap® are registered trademarks, and NucleoVac™ is a trademark, of Macherey-Nagel GmbH & Co. ProLong® is a registered trademark of Molecular Probes. QuickClean™ is a trademark of Clonogene, Inc. SpectraMAX Plus® is a registered trademark of Molecular Devices. Superflow™ & Uniflow™ are trademarks of Sterogene Bioseparations, Inc.

In the October 1998 issue, we neglected to state that FPLC® and Sepharose® are registered trademarks of Amersham Pharmacia Biotech. We apologize for this oversight.

BD
1 Becton Drive
Franklin Lakes, NJ USA 07417

BD Biosciences Clontech
1020 East Meadow Circle
Palo Alto, CA 94303
877.232.8995, orders@clontech.com

NEW PRODUCTS

ApoAlert™ CPP32 Protease Assay Kits

Detect ICE-family protease activity by fluorometric or colorimetric methods

- **Assay one of the earliest indicators of apoptosis**
- **Quick, quantitative assay performed on cell lysates**
- **Detect an essential event in apoptosis—regardless of induction method or cell type**
- **Can be easily formatted in microtiter plates for high throughput**

CLONTECH's **ApoAlert™ CPP32 Assay Kits**, the first in a line of apoptosis-related protease assays, provide a simple and convenient means for assaying CPP32 protease activity, a key early event in apoptosis. These fluorometric and colorimetric kits are based on the detection of molecules cleaved from specific protease substrates and are the first commercial kits for quantifying the activity of an ICE-family protease.

Two detection methods

The CPP32 Assay Kit is available in two versions depending on the desired method of detection: fluorometry or colorimetry. Both methods take advantage of the same straightforward assay principle, incorporating speed, convenience, and high sensitivity. Figure 1 shows the cleavage reactions central to each detection method. The ApoAlert CPP32 Fluorescent Assay Kit detects the shift in fluorescence emission of the molecule 7-amino-4-trifluoromethyl coumarin (AFC). The AFC-substrate conjugate, DEVD-AFC, emits blue light (λ_{max} = 400 nm). However, upon proteolytic cleavage of the substrate by CPP32, the free AFC emits a yellow-green fluorescence at 505 nm. Comparison of the fluorescence from an apoptotic sample with an uninduced control allows quantification of the increase in protease activity.

Similarly, the ApoAlert CPP32 Colorimetric Assay is based on spectrophotometric detection of the chromophore *p*-nitroanilide (pNA) after

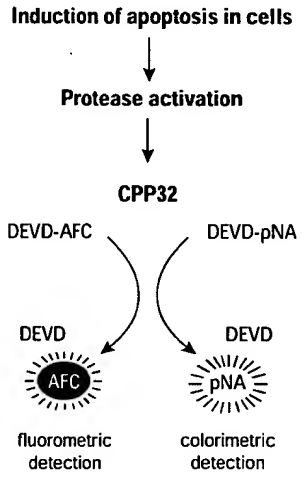

Figure 1. Methods of detection of protease activity. The ApoAlert CPP32 Assay Kit is available in fluorometric and colorimetric formats. Fluorometric detection is performed at 505 nm; colorimetric detection is performed in a spectrophotometer at 405 nm.

The Role of Proteases in Apoptosis

Apoptosis, or programmed cell death, plays a fundamental role in many normal biological processes as well as several disease states (1). Apoptosis can be induced by various stimuli that all produce the same end result: systematic and deliberate cell death. ICE (interleukin-1β converting enzyme; 2) is the mammalian homolog of the *C. elegans ced-3* gene, one of several "death genes" required for somatic cell death in nematode development. While the role of ICE in apoptosis induction is not clear, other ICE family members are known to play key, early roles in this process (for review, see reference 3). One of these proteases, CPP32 (4), plays a direct role in the proteolytic digestion of cellular proteins responsible for progression to apoptosis (see Figure 2).

All the ICE family members are cysteine proteases that possess the unusual ability to cleave substrates after aspartate residues. This activity is central to their role in mammalian apoptosis. In addition to activating other proteases, CPP32 and other ICE family members are themselves activated by cleavage processes at these same aspartate residue sites. The details of this complex autoregulation remain to be elucidated.

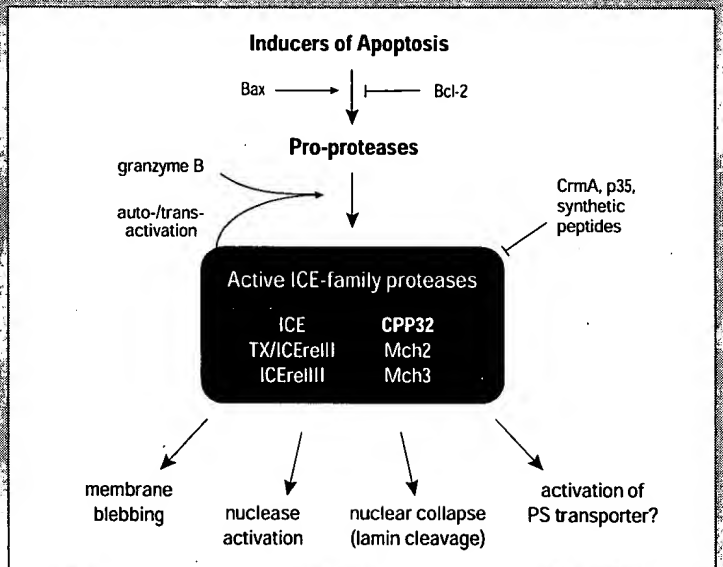

Figure 2. Apoptosis can be triggered by both environmental and developmental cues. The ICE family of proteases is responsible for the specific cleavage of a set of structural and regulatory proteins which leads to cell death. Positive and negative factors act on the pathway at several points to regulate protease activity.

(800) 662-CLON (2566) • (415) 424-8222 ■ FAX (800) 424-1350 • (415) 424-1064
e-mail: tech@clontech.com • orders@clontech.com

ApoAlert™ CPP32 Protease Assay Kits...*continued*

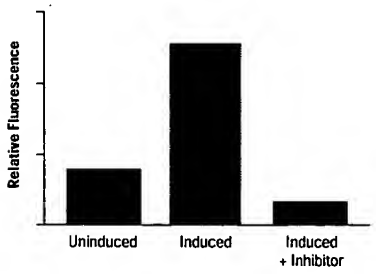

Figure 6. Inhibition by DEVD-CHO, a specific inhibitor of CPP32 activity. 32D cells were grown in the presence (uninduced) or absence (induced) of 2.5 ng/ml IL-3. Inhibitor was added to the indicated sample; samples were processed as described in Figure 3.

microscopy. The CPP32 Assay Kits offer a low-cost, quantitative assay to researchers without a FACS machine, and the CPP32 Colorimetric Assay Kit permits early detection of apoptosis using a spectrophotometer. The CPP32 assay is also better suited to analysis of tissue samples since intact cells are not required.

The ApoAlert CPP32 Assay Kits are each available in two convenient sizes, for either 25 or 100 assays. Each kit includes labeled protease substrate (DEVD-AFC for the fluorescent assay and DEVD-pNA for the colorimetric assay), CPP32 Inhibitor, free label for use as a standard (AFC or pNA), the necessary buffers and reagents, and a complete User Manual (PT3083-1).

References

1. Thompson, C. B. (1995) *Science* **267**:1456–1562.
2. Yuan, J., *et al.* (1993) *Cell* **75**:641–652.
3. Nicholson, D. W. (1996) *Nature Biotechnol.* **14**: 297–301.
4. Fernandes-Alnemri, T., *et al.* (1994) *J. Biol. Chem.* **269**:30761–30764.
5. Lazebnik, Y. A., *et al.* (1994) *Nature* **371**:346–347.
6. Casciola-Rosen, L., *et al.* (1994) *J. Biol. Chem.* **269**: 30757–30760.
7. Casciola-Rosen, L., *et al.* (1996) *J. Exp. Med.* **183**: 1957–1964.
8. Apoptosis-Inducing Reagents (October 1996) *CLONTECHniques* **XI**(4):14–15.
9. Martin, S. J., *et al.* (1995) *J. Exp. Med.* **182**: 1545–1556.
10. ApoAlert Annexin V Apoptosis Kit (July 1996) *CLONTECHniques* **XI**(3):9–11.

Product	Size	Cat. #
ApoAlert CPP32 Fluorescent Assay Kit	25 assays 100 assays	K2026-1 K2026-2
ApoAlert CPP32 Colorimetric Assay Kit	25 assays 100 assays	K2027-1 K2027-2
ApoAlert CPP32 Inhibitor, DEVD-CHO	100 µl	8170-1
ApoAlert ICE Inhibitor, YVAD-CMK	100 µl	8171-1

ApoAlert™ CPP32 Assay Kit Components

- **Cell Lysis Buffer**
- **2X Reaction Buffer**
- **DTT**
- **CPP32 Fluorescent or Chromogenic Substrate (DEVD-AFC or DEVD-pNA)**
- **CPP32 Inhibitor, DEVD-CHO**
- **Free Fluorophore or Chromophore (AFC or pNA)**
- **Complete User Manual** (PT3083-1)

Related Products

- **ApoAlert™ Annexin V Apoptosis Kit** (#K2025-1, -2)
- **Actinomycin D** (#8153-1)
- **Anti-Fas (clone Dx2)** (#8150-1)
- **C_2-Ceramide** (#8152-1)
- **Cycloheximide** (#8158-1)
- **Dexamethasone** (#8155-1)
- **Etoposide** (#8154-1)
- **Human TNF-α** (#8157-1)
- **Staurosporine** (#8151-1)
- **Vincristine Sulfate** (#8156-1)

LexA Monoclonal Antibody

The **LexA Monoclonal Antibody** (mAb) is a valuable tool for characterizing LexA fusion proteins generated with the high-expression pLexA plasmid (originally published as pEG202; 1) provided in the MATCHMAKER LexA Two-Hybrid System (#K1609-1; 2). Probing Western blots of yeast extracts with this mAb yields a single band (Figure 1). The LexA mAb can be used to confirm that LexA fusions to known proteins are being expressed and have the expected molecular weight before being used in a two-hybrid assay. Quantification can be performed on dot blots if an appropriate, purified control protein is available.

The LexA mAb is purified from the serum-free medium of mouse hybridoma cultures. Sufficient mAb is provided with each order to probe ten 50-cm² Western blots. A complete User Manual (PT1029-1) and the CLONTECH Yeast Protocols Handbook (PT3024-1) are included.

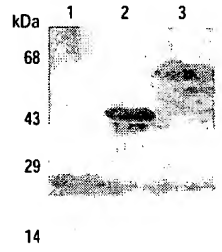

Figure 1. Western blot using the LexA mAb. Soluble protein extracts (3) were prepared from yeast strain EGY48[p8op-lacZ] transformed with the indicated plasmid. Samples equivalent to ~1–1.5 OD_{600} units of cells were resolved by SDS-PAGE and electroblotted to a nitrocellulose filter. The blot was probed with LexA mAb (20 ng/ml) using 1 ml of diluted mAb per 10 cm² of blot, followed by AP-conjugated secondary antibody. Signals were detected using a BCIP/NBT substrate. Lane 1: LexA protein expressed from pLexA; 25.5 kDa. Lane 2: pLexA-Lam encodes a 44.4-kDa fusion of LexA with human lamin C. Lane 3: pLexA-53 encodes a 58.2-kDa fusion of LexA with murine p53.

Product	Size	Cat. #
LexA Monoclonal Antibody	4 µg	5397-1

Available February 1, 1997.

Related Products

- **MATCHMAKER LexA Two-Hybrid System** (#K1609-1)
- **MATCHMAKER LexA Libraries** (many; see page 17)
- **MATCHMAKER B42AD LD-Insert Screening Amplimer Set** (#9108-1)
- **MATCHMAKER LexA DNA-BD Insert Screening Amplimer Set** (#9109-1)
- **GAL4 AD & DNA-BD Monoclonal Antibodies** (#5398-1, #5399-1)

References

1. Gyuris, J., *et al.* (1993) *Cell* **75**:791–803.
2. MATCHMAKER LexA Two-Hybrid System (July 1996) *CLONTECHniques* **XI**(3):14–17.
3. Printen, J. A. & Sprague, Jr., G. F. (1994) *Genetics* **138**:609–619.

ApoAlert® Caspase-9/6 Fluorescent Assay Kit

Detect caspase-9/6 activity by fluorometric methods

- **Assay the first known caspase activated via the mitochondrial apoptotic pathway**
- **Obtain quantitative results in 90 minutes**
- **Can be formatted for high-throughput analysis**

CLONTECH's **ApoAlert® Caspase-9/6 Fluorescent Assay Kit** provides a simple way to detect caspase-9 and -6 activation. These caspases are both activated via mitochondrial involvement in the apoptotic pathway, making this kit very useful for investigating the role of mitochondria in apoptosis.

Cells exposed to apoptotic stimuli release cytochrome c from mitochondria into the cytosol. In the cytosol, cytochrome c interacts with apoptotic protease activating factor-1 (Apaf-1; 1). The cytochrome c/Apaf-1 complex cleaves the inactive caspase-9 proenzyme to generate the active enzyme (2). Activated caspase-9 then initiates the proteolytic activities of other downstream caspases, including caspase-3 and caspase-6. These caspases degrade a variety of substrates, resulting in the systematic disintegration of the cell.

Simple, straightforward detection method

The Caspase-9/6 Assay Kit uses the same principle as our other caspase detection kits: you can quickly detect caspase activation by assaying for the cleavage of a fluorescent substrate. The kit uses the substrate LEHD-AMC, which is cleaved by both caspase-9 and caspase-6 (Figure 1). When LEHD-AMC is cleaved, the released AMC molecule fluoresces green. By comparing the fluorescence from an apoptotic sample and an uninduced control, you can quantify the increase in caspase activity.

Because caspase-9 occupies an upstream position in the caspase cascade, only a small amount of activated caspase-9 is needed to trigger downstream events. Thus, the difference in activity between uninduced and induced cells detected by this kit is relatively small in comparison to the results obtained by using CLONTECH's Caspase-3 and Caspase-8 Assay Kits. Caspase-9 activity also varies in different cell types. In Figure 2A, we used the Caspase-9/6 Assay Kit to monitor apoptosis in Jurkat cells treated with Fas Antibody. Induced cells exhibited a 4-fold increase in caspase-9 activity compared with uninduced cells. The NIH/3T3 cells shown in Figure 2B were treated with staurosporine overnight and showed an approximate two-fold increase in caspase-9 activity over uninduced cells.

The ApoAlert Caspase-9/6 Assay Kit is available in 25- or 100-assay sizes. Each kit includes Caspase 9/6 Substrate (LEHD-AMC), Caspase-9 Inhibitor, the necessary buffers and reagents, and a complete User Manual.

See facing page for ordering information.

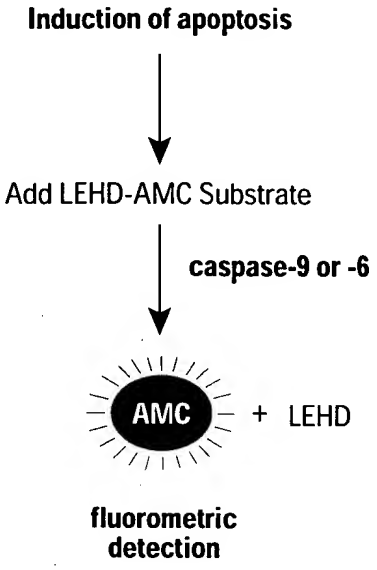

Figure 1. Fluorometric detection of protease activity. The ApoAlert Caspase-9/6 Fluorescent Assay Kit detects the shift in fluorescence emission of the molecule 7-amino-4-methoxy coumarin (AMC), which is conjugated to the tetrapeptide LEHD. Upon cleavage, AMC fluorescence can be measured using a 380-nm excitation filter and a 460-nm emission filter.

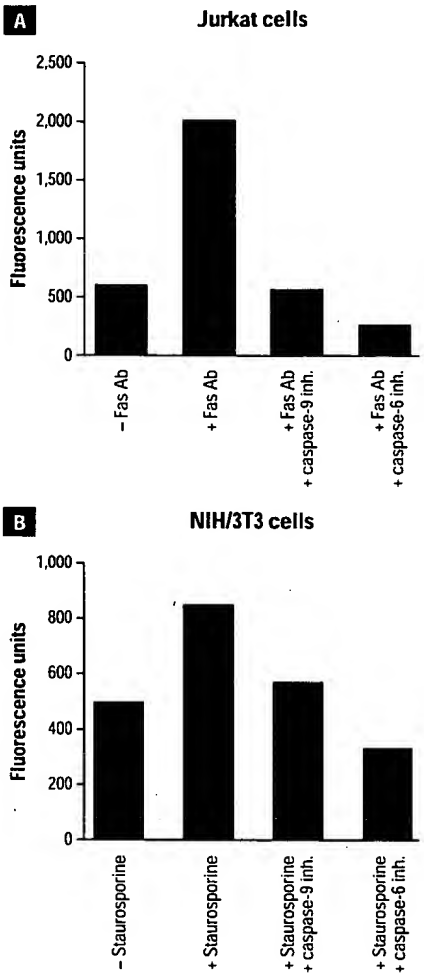

Figure 2. Detection of caspase-9/6 activity in Jurkat and NIH/3T3 cells. LEHD-AMC is cleaved by both caspase-9 and -6. Apoptosis was induced as indicated. Cells were harvested and lysates were assayed as described in the User Manual. **Panel A.** Jurkat cells were incubated with or without Fas antibody (500 ng/ml) for 6 hr. Lysates were prepared and incubated with the caspase-9 substrate with or without caspase-9 or caspase-6 inhibitors. **Panel B.** NIH/3T3 cells incubated with or without staurosporine (300 nM) overnight. Lysates were prepared and incubated with the caspase-9 substrate either with or without caspase-9 or caspase-6 inhibitors.

ApoAlert® Cell Fractionation Kit

Isolate a mitochondria-enriched fraction from the cytosol without ultracentrifugation

- **Determine the apoptotic pathway in your model system**
- **Faster & easier than ultracentrifuge-based methods**
- **Use mitochondria-enriched fraction for downstream applications**

CLONTECH's **ApoAlert® Cell Fractionation Kit** provides an effective way to isolate a highly enriched mitochondrial fraction from the cytosol of apoptotic and nonapoptotic cells. With this kit, you can easily determine if cytochrome c has been released from mitochondria and is present in the cytosolic fraction, an indicator of mitochondrial involvement in apoptosis. The procedure is fast and simple—no ultracentrifugation is required.

Relocation of cytochrome c

Cytochrome c has been shown to play a major role in apoptosis (1, 2). This soluble protein is localized in the space between the inner and outer mitochondrial membranes. An apoptotic stimulus triggers the release of cytochrome c from the mitochondria into the cytosol, where it initiates the caspase cascade by binding to Apaf-1. The cytochrome c/Apaf-1 complex activates caspase-9, which then activates caspase-3 and other downstream caspases.

Isolate mitochondrial fraction

The ApoAlert cell fractionation protocol involves just two standard centrifugation steps to separate a mitochondria-enriched fraction from cytosol. The antibodies included in the kit allow you to distinguish between the fractions. Cytochrome c oxidase subunit IV (COX4), a membrane protein localized in the inner mitochondrial membrane, remains in the mitochondria during apoptosis. The COX4 Antibody is thus a useful marker for the mitochondria-enriched fraction. Figure 3 shows a Western blot of mitochondrial and cytosolic fractions probed with the COX4 Antibody, showing an efficient separation of mitochondria from cytosol.

With the Cytochrome c Antibody, you can determine the location of cytochrome c by probing Western blots of samples of cytosolic and mitochondria fractions. A positive result for the cytosolic fraction indicates that cytochrome c was released from the mitochondria. Figures 3B and 3C show cytosolic and mitochondria fractions from induced and uninduced cells. Cytochrome c was detected in the cytosol of induced cells.

The Cell Fractionation Kit provides optimized reagents and antibodies sufficient for 100 assays.

NEW!

Product	Size	Cat.#
ApoAlert Caspase-9/6 Fluorescent Assay Kit	100 assays 25 assays	K2015-1 K2015-2
ApoAlert Cell Fractionation Kit	100 assays	K2016-1

ApoAlert® Caspase-9/6 Fluorescent Assay Kit Components

- **Cell Lysis Buffer**
- **Reaction Buffer**
- **DTT**
- **Caspase-9/6 Substrate** (LEHD-AMC)
- **Caspase-9 Inhibitor** (LEHD-CHO)
- **DMSO**
- **Complete User Manual** (PT3191-1)

ApoAlert® Cell Fractionation Kit Components

- **Cell Fractionation Buffer**
- **Cell Wash Buffer**
- **Protease Inhibitor**
- **DTT**
- **Cytochrome c Antibody**
- **COX4 Antibody**
- **Protocol-at-a-Glance** (PT3324-2)

Related Products

- **ApoAlert® Mitochondrial Membrane Sensor Kit** (#K2017-1)
- **ApoAlert® Caspase-3 Assay Kits**
  Fluorescent (#K2026-1, -2)
  Colorimetric (#K2027-1, -2)
- **ApoAlert® Caspase-8 Assay Kits**
  Fluorescent (#K2028-1, -2)
  Colorimetric (#K2029-1, -2)
- **ApoAlert® Apo 2.7/Annexin V-EGFP Kit** (#K2018-1)
- **ApoAlert® Annexin V-EGFP Apoptosis Kit** (#K2019-1, -2)
- **ApoAlert® Annexin V-FITC Apoptosis Kit** (#K2025-1, -2)
- **ApoAlert® LM-PCR Ladder Assay Kit** (#K2021-1)
- **ApoAlert® DNA Fragmentation Assay Kit** (#K2024-1, -2)
- **Apoptosis inducing agents** (many)

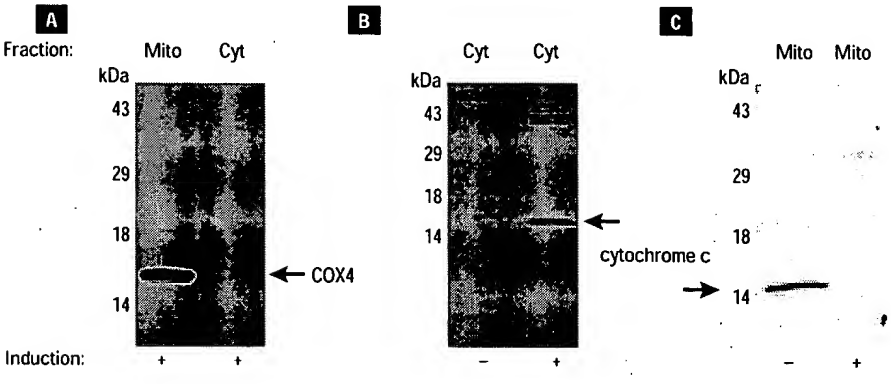

Figure 3. Western blot detection of cytochrome c oxidase subunit IV and cytochrome c. Apoptosis was induced with staurosporine in NIH/3T3 cells. Six hr after induction, the mitochondrial and cytosolic fractions were isolated using the ApoAlert Cell Fractionation Kit. **Panel A.** Mitochondrial and cytosolic fractions from induced cells; probed with COX4 Antibody. **Panel B.** Cytosolic fractions from induced and uninduced cells; probed with Cytochrome c Antibody. **Panel C.** Mitochondrial fractions from induced and uninduced cells; probed with Cytochrome c Antibody.

References

1. Liu, X., *et al.* 1996 *Cell* 86:147–157.
2. Peng, L., *et al.* 1997 *Cell* 91:479–489.

TEL: 800-662-2566 (CLON) • 650-424-8222 ■ INTERNET: www.clontech.com
E-MAIL: products@clontech.com • orders@clontech.com